stranded RNA. During replication of the retroviral particle, a retroviral enzyme (reverse transcriptase) makes a double stranded DNA (dsDNA) copy of the viral RNA molecule and removes the RNA. Another viral enzyme (integrase) catalyzes the integration of the dsDNA into the chromosome of the host cell. An enzyme of the host cell (RNA polymerase) then transcribes its host cell DNA (into which is integrated the viral genome) thereby making RNA copies of the viral genome which are then assembled into viral particles and released from the cell by a process of budding (Essential Cell Biology: An Introduction to the Molecular Biology of the Cell, Bruce Alberts *et al.* (1998) being filed herewith as Exhibit A). Upon review of the claims, Applicants realized that "a recombinant retroviral particle, said particle comprising ***a DNA sequence*** encoding SDI-1. . ." was not technically accurate. The retroviral particle has an RNA genome, and a DNA copy of the retroviral particle's RNA genome does not occur until the retroviral particle infects a host cell. Therefore, in the previously filed Amendment mailed to the U.S. Patent Office on October 11, 2001, Applicants amended the claimed invention to more accurately reflect the life cycle of the retroviral genome.

Applicants teach that the RNA genome of the recombinant retroviral particle (which is not in an antisense orientation) can comprise an RNA sequence which encodes ***SDI-1*** sequences 1) in the ***sense*** orientation, which inhibits cell proliferation and 2) in the ***antisense*** orientation, which enhances cell proliferation (specification, page 4, lines 15-18). Applicants teach that:

> Antisense sequences are nucleic acids (either DNA or RNA) whose sequence is complementary to the sequence of a target mRNA molecule (or its corresponding gene) so that it is capable of hybridizing with/binding to the mRNA molecule (or gene) and thereby impairing (i.e. attenuating or preventing) the transcription of the gene into mRNA or the translation of the mRNA molecule into a gene product (specification, page 3, lines 14-18).

That is, antisense RNA is an RNA molecule:

> generated by reversing the orientation of the transcribed region of a gene [*e.g.*, SDI-1] with respect to a suitable transcriptional PROMOTER. This results in generating a transcript of the ANTISENSE DNA strand. Such antisense RNA has the potential to form an RNA-RNA duplex with the natural 'sense' mRNA transcript of the gene, ***thereby preventing its translation*** (The Encyclopedia of Molecular Biology, Blackwell Science (1994), page 66, emphasis added a copy of which is being filed as Exhibit B).

As the Examiner notes, Applicants elected a recombinant retroviral particle comprising an RNA sequence which encodes ***SDI-1*** sequences in the ***sense*** orientation, which when expressed, inhibits cell proliferation. The RNA genome of a retroviral particle and the SDI-1 sequence of the elected species are in the sense orientation, and thus, are translated by the infected host cell.

Claims 1-4, 9-11, 33-38, 44 and 49 are ***not*** directed to retroviruses comprising antisense. The elected claims have been searched and there is no need for an additional search. Applicants respectfully request withdrawal of the most recent restriction and re-entry of Claims 1-4, 9-11, 33-38, 44, 49, 54 and 55 into the elected species invention of retroviruses comprising sense nucleic acids.

Rejection of Claims 13-16, 19-23, 26-28, 31, 32, 41, 42, 45-48, 50-53 and 56-61 under 35 U.S.C. §112, first paragraph

Claims 13-16, 19-23, 26-28, 31, 32, 41, 42, 45-48, 50-53 and 56-61 are rejected under 35 U.S.C. §112, first paragraph because the specification "does not reasonably provide enablement for fragments or analogs of SDI-1, treating any disease, treating any symptom of cancer or restenosis, using any mode of delivery, capsules comprising producer cells, methods of using such capsules, pharmaceutical compositions comprising producer cells, or methods of using such pharmaceutical compositions" (Office Action, page 3). The Examiner states that Crystal and Feldman "taught the combination of vector and mode of delivery for gene therapy required to target the desired tissue and provide adequate expression of a protein such that a desired effect was obtained was unpredictable" and that Nabel "taught administering a viral vector encoding SDI-1 to a restenosis patient by catheter or direct injection into a blood vessel at the site of the restenosis resulting in a decrease in the intimal hyperplasia in said blood vessel" (Office Action, page 4). The Examiner concludes that the "art at the time of filing did not teach how to administer retroviral packaging cells of capsules comprising retroviral packaging cells to patients such that a therapeutic effect was obtained"; that "it was unpredictable how to administer retroviral packaging cells or capsules comprising retroviral packaging cells to patients such that a therapeutic effect was obtained" and that "one of skill in the art at the time the invention was made would have been limited to using retroviral particles encoding SDI-1 to treat restenosis as taught by Nabel" (Office Action, page 4). The Examiner states that "Applicants have not

correlated administering retroviral particles encoding SDI-1 for treating restenosis as taught by Nabel to administering packaging cells or capsules such that the same tissue is targeted, equivalent amounts of retroviral particles are contacted with the tissue or that a therapeutic effect is obtained" (Office Action, pages 4-5).

Applicants respectfully disagree. The Examiner is not considering Applicants' *in vitro* data in light of what is known to those of skill in the art. Furthermore, Applicants fail to understand how Applicants' invention can be obvious based on the teachings of Nabel if "one of skill in the art at the time the invention was made would have been limited to using retroviral particles encoding SDI-1 to treat restenosis as taught by Nabel" (Office Action, page 4).

As pointed out in the previously filed Amendments, Applicants demonstrate that a human bladder carcinoma derived cell line transfected with a gene encoding SDI-1 "showed significantly more cells in G_0/G_1 when grown in the presence of Dex after serum starvation (61%) than in the absence of SDI-1 expression (50.6%)" (specification, page 26, lines 20-22). The Examiner is not considering Applicants' *in vitro* data ***in combination with*** the *in vitro* data of Nabel showing that expression of p21 inhibited vascular cell proliferation and induced cell cycle arrest *in vitro* (Nabel *et al.*, column 7, line 59 - column 8, line 60) and that these *in vitro* results correlated to results they received *in vivo* (Nabel *et al.*, column 8, line 61 - column 11, line 7); the *in vivo* data of Price *et al.* demonstrating "the utility of retrovirus vectors for the introduction of genes into neural cell precursors" (Price *et al.*, page 160, column 1); and the teaching of Miller *et al.* that retroviruses "will be useful for the treatment of humans" (Miller *et al.*, page 989, column 3). It is reasonable to expect based on Applicants' data, ***and with the knowledge attributed to those of skill in the art*** (as indicated in the art of record), that producer cells stably transfected with a retroviral particle comprising a DNA sequence encoding SDI-1, retroviral particles produced by such a cell line and capsules which encapsulate such producer cell lines can be administered to an individual for the treatment of diseases or disorders responsive to the anti-proliferative activity of SDI-1.

Furthermore, Crystal and Feldman do not teach that "the combination of vector and mode of delivery for gene therapy required to target the desired tissue and provide adequate expression of a protein such that a desired effect was obtained was unpredictable" (Office Action, page 4). For example, Crystal clearly teaches that:

> Enough information has been gained from clinical trials to allow the conclusion that human gene transfer is feasible, can evoke biologic responses that are relevant to human disease, and can provide important insights into human biology. Adverse events have been uncommon and have been related to the gene delivery strategies, not to the genetic material being transferred (Crystal, abstract).

Feldman *et al.* review the "[r]esults of the principal clinical trials and new avenues for protection of restenosis" (Feldman *et al.*, page 9, column 1). However, as the Examiner notes, Nabel teach that "administering a viral vector encoding SDI-1 to a restenosis patient by catheter or direct injection into a blood vessel at the site of the restenosis resulting in a decrease in the intimal hyperplasia in said blood vessel" (Office Action, page 4).

The court has clearly stated that:

> Enablement is not precluded by the necessity for experimentation such as routine screening... However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue' not 'experimentation'... The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness having due regard for nature of the invention and the state of the art... The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed (In re Wands, 1400 U.S.P.Q.2d 1400, 1404 (CAFC 1988)).

Although some experimentation may be necessary to practice Applicant's claimed invention, such experimentation is not undue. As pointed out above, Applicants have provided sufficient guidance for carrying out the claimed invention to those of skill in the art. Applying a standard of reasonableness having due regard for nature of the invention and the state of the art, it is clear that those of skill in the art would not find the experimentation to practice Applicants' claimed invention undue. Applicants again direct the Examiner's attention to the teaching by those of skill in the art that "human gene transfer is feasible, can evoke biologic responses that are relevant to human disease, and can provide important insights into human biology" and that "[a]dverse events have been uncommon and have been related to the gene delivery strategies, not to the genetic material being transferred" (Crystal, abstract). However, Applicants use retroviral vectors to deliver the SDI-1 sequence. As also noted above, Price *et al.* demonstrate "the utility of retrovirus vectors for the introduction of genes into neural cell precursors" *in vivo* (Price *et al.*,

page 160, column 1); and Miller *et al.* teach that retroviruses "will be useful for the treatment of humans" (Miller *et al.*, page 989, column 3). Clearly, Applicants have provided an enabling disclosure for treating a disease, such as cancer or restenosis, comprising administering retroviruses which encode, and thereby deliver, SDI-1 to the cells of an individual.

Regarding analogues or functional fragments of SDI-1, the Examiner states that he "cannot determine by eye that amino acids 1-71 of El-Deiry, Harper and Xiong are 100% identical" and that "the specification does not provide an assay for determining fragments of SDI-1 that have the same function as full length SDI-1" (Office Action, page 5). The Examiner states that "the specification does not teach amino acids 42-58 has equivalent biological activity as full length SDI-1" (Office Action, page 5). The Examiner further states that the specification "does not teach an assay to determine whether the mutants have the desired function" (Office Action, pages 5-6).

Applicants respectfully disagree. As noted in the previously filed Amendment mailed to the U.S. Patent Office on October 11, 2001, citing the WAF1 sequence of El-Deiry (*Cell, 75:*817-825 (1993) (Reference AR2 of PTO 1449), Hunter notes that the "Sequence of ***Waf1 is identical to Cip1***" (*Cell, 75:*839-841 (1993), Reference AT2 of PTO 1449, page 840, column 1, emphasis added). Citing the WAF1 sequence of El-Deiry, Harper also notes that the "***WAF1 is identical to CIP1***" (*Cell, 75:*805-816 (1993), Reference AZ of PTO 1449, page 813, column 2, emphasis added). Applicants' Attorney has compared the 1-71 amino acids of the WAF1 sequence of El-Deiry with the p21 amino acid sequence of Xiong *et al.*, and the sequences are indeed identical.

Furthermore, Applicants have provided an assay to determine whether analogues, functional fragments or mutants of SDI-1 have the desired function of inhibiting cell proliferation. As noted previously, Applicants demonstrate that a human bladder carcinoma derived cell line transfected with a gene encoding SDI-1 "showed significantly more cells in G_0/G_1 when grown in the presence of Dex after serum starvation (61%) than in the absence of SDI-1 expression (50.6%)" (specification, page 26, lines 20-22). Thus, using this same assay one of skill in the art can transfect a human cell line with a gene encoding an analogue, functional fragment or mutant of SDI-1 and determine whether the presence of the analogue, functional fragment or mutant causes the cells to be in the G_0 G_1 phase, which is an indicator of inhibition

of cell proliferation. Finally, there is no requirement that amino acids 42-58 of SDI-1 have equivalent biological activity as full length SDI-1. However, Applicants have clearly provided an assay for determining whether amino acids 42-58 of SDI-1 inhibits cell proliferation.

Applicants have provided an enabling disclosure for the full scope of the claimed invention.

Rejection of Claims 31 and 32 under 35 U.S.C. §112, second paragraph

Claims 31 and 32 are rejected under 35 U.S.C. §112, second paragraph "as bing indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 6). The Examiner states that "[i]f applicants intend the claims to encompass administering retroviral particles or packaging cells producing retroviral particles, then parent claims should not be limited to administering retroviral particles" (Office Action, page 6). The Examiner further states that "[a]dministering retroviral particles in parent claim 27 does not encompass administering packaging cells producing retoviral particles" and "[i]f cells are administered, retroviral particles are produced *in vivo*, they are not 'administered'" (Office Action, page 6).

Claims 31 and 32 have been amended to more clearly indicate a method of administering a retroviral particle and a producer cell line, respectively.

Rejection of Claims 13, 14, 19, 26-28, 31, 32, 39, 40, 45, 48, 50 and 53 under 35 U.S.C. §103(a)

Claims 13, 14, 19, 26-28, 31, 32, 39, 40, 45, 48, 50 and 53 are rejected under 35 U.S.C. §103(a) as being unpatentable over Miller or Price in view of Nabel. The Examiner has considered Applicants arguments, but has found them unpersuasive "because Nabel taught packaging cells producing viral particles encoding SDI-1 produced viral particles (col. 6, line 22)" (Office Action, page 8). The Examiner concludes that "one of ordinary skill would have recognized that packaging cells producing retroviral particles encoding SDI-1 would not be severely damaged or be unable to produce viral particles because of SDI-1 expression" (Office Action, page 8).

Applicants respectfully disagree. As pointed out in the previously filed Amendments, Nabel *et al.* used an adenoviral DNA vector comprising the p21 gene and the genes required for

synthesis of packaging proteins and in which the E1A and E1B replicases and activators of gene expression were deleted. The deleted E1A and E1B were provided by the 293 cell line used for propagation of recombinant adenoviruses (Nabel *et al.*, column 6, lines 22-34). Generation of ***DNA viruses*** involve the packaging of the DNA with proteins provided in the vector, and release of the DNA viruses by ***lysis of the packaging cell***. During transient transfection, the vector-DNA in both DNA and RNA viruses is transient, not stably present in the packaging cell line, *i.e.*, during this period of transient transfection, the foreign vector-DNA is degraded by DNAases included in the packaging cell line and the vector-DNA is ***not*** transmitted to ***daughter packaging cells*** during division of the packaging cells. With DNA virus-derived vectors, the packaging cells are lysed upon release of the DNA viruses.

However, with ***RNA virus-derived vectors***, the transfected vector-DNA is integrated into the packaging cell genome resulting in stably transfected producer cells, since it is also a common characteristic of retroviruses to integrate into the genome of cells. As a result, ***during cell division daughter cells of the parental packaging cells are provided with the recombinant vector-DNA. In this case, daughter cells also become packaging cells for their recombinant DNA, resulting in a stable population of RNA-virus producing cells***. With RNA viruses, the genome-integrated recombinant DNA must at first be transcribed into RNA. ***The transcribed recombinant retroviral RNA is not only simply packaged into proteins but also serves as a template for further translation***, *i.e.*, the retroviral RNA is recognized by the translation machinery of the packaging cell as mRNA. This is not the case with the adenoviral DNA vector of Nabel *et al.*

The Examiner states that "Nabel suggested making packaging cells producing retroviral particles (col. 3, lines 10) and has a claim that encompasses treating restenosis using retroviral particles encoding SDI-1 (claim 1)" (Office Action, page 8).

Applicants respectfully disagree. Nabel *et al.* state that:

> Viral vectors have been indicated as highly efficient in transferring genes to mammals containing deficient genes . . . Preferably, retroviral vectors with impaired ability to replicate and transform are used. ***Suitable viral vectors which express p21 useful in accordance with the present invention include adenoviral vectors, Ad5-360 in combination with pAD-BgIII . . . . Preferably adenoviral vectors are used*** (Nabel *et al.*, column 3, lines 5-16).

Nabel *et al.* clearly state that "[s]uitable viral vectors" for use in the present invention are "adenoviral vectors", ***not*** retroviral vectors. Thus, when reading Claim 1 of the Nabel *et al.* patent in light of the teachings in the specification, it is clear that Claim 1 encompasses use of adenoviral vectors, but does ***not*** encompass use of retroviral vectors.

Miller *et al.* and Price *et al.* do not provide what is lacking in the Nabel *et al.* reference. Miller *et al.* "designed a set of retroviral vectors which cannot yield helper virus by homologous recombination with the retroviral genome present in the packaging cells, and include mutations to block viral protein synthesis" (Miller *et al.*, page 980, column 3). Price *et al.* demonstrate that "retroviruses that encode the β-gal gene can be successfully introduced into the rat retina and mark cells such that they can easily be detected histochemically" (Price *et al.*, page 156, column 2).

However, as pointed out in the previously filed Amendments, a person of skill in the art would not be motivated to combine the teachings of Nabel *et al.* with the teachings of Miller *et al.* or Price *et al.* to produce Applicants' claimed invention because one of skill would not expect that a ***stably transfected producer cell line comprising a retroviral genome which encodes the SDI-1*** could be produced. When including an SDI-1 gene into a retroviral vector, the SDI-1 gene is transcribed and translated into protein. However, SDI-1 is known to inhibit cell proliferation and DNA synthesis, and thus, prevent cell division. Accordingly, a person of skill in the art would not expect to get a stable population of retrovirus producing cells (***a stable population of daughter packaging cells***) by stable integration of a recombinant retroviral vector comprising the SDI-1 gene. Rather, a person of skill in the art would expect that after integration of the retroviral vector into the genome of the packaging cell, division of the cell would be inhibited by virtue of the expressed SDI-1 protein, and thus, ***stable daughter packaging cells*** which produce RNA-virus would not be generated. However, Applicants have shown that stable populations of recombinant retroviral particles producing cells stably transfected with a retroviral vector comprising SDI-1 are generated.

The teachings of Miller *et al.*, Price *et al.*, Nabel *et al.* and Haertig *et al.* do not render obvious Applicants' claimed invention.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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Dated: December 23, 2002

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

13. (Four times amended) An isolated producer cell line stably transfected with a retroviral vector comprising a DNA sequence encoding SDI-1, a functional analogue thereof, or a functional fragment thereof, wherein the SDI-1 or functional fragment or functional analogue thereof inhibits cell proliferation, said producer cell line additionally harboring at least one DNA construct coding for the proteins required for said retroviral vector to be packaged.

31. (Twice amended) A method according to Claim 28 wherein the recombinant retroviral particle is administered as an injection[, or the recombinant retroviral particle is administered by implantation of the producer cell line harbouring:

a) a retroviral vector carrying a DNA sequence encoding SDI-1, a functional analogue, a fragment thereof or an antisense SDI-1 DNA sequence; and

b) at least one DNA construct coding for the proteins required for said retroviral vector to be packaged]

into the living animal body, including a human, nearby or at the site of the tumor.

32. (Twice amended) A method for the treatment of a disorder or disease responsive to the antiproliferative activity of SDI-1 comprising administering a producer cell line according to Claim [28] 13 [wherein the recombinant retroviral particle is administered as an injection, or the recombinant retroviral particle is administered by implantation of an encapsulated packaging cell line comprising encapsulated cells having a core containing packaging cells harbouring:

a) a retroviral vector carrying a DNA sequence encoding SDI-1, a functional analogue, a fragment thereof or an antisense SDI-1 DNA sequence; and

b) at least one DNA construct coding for the proteins required for said retroviral vector to be packaged

and a porous capsule wall surrounding said core, said porous capsule wall being permeable to the retroviral particles produced by the packaging cells, into the living animal body, including a human, nearby or at the site of the tumor].